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EVALUATION OF A NEWLY MODIFIED LIQUID MEDIUM FOR CULTIVATION OF TRICHOMONAS VAGINALIS

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For demonstration of *Trichomonas vaginalis*, commercial liquid media commonly used in most clinical laboratories but none has proven to be fully satisfactory in respect of preservation, trichomonad detectability and price.

Experiments were performed to assess usefulness of a trial preparation of liver extract-free medium. The results indicate that the new medium is superior to the conventional media with respect to cost and preservability but there is no appreciable difference in trichomonad detectability between them.

Key words *Trichomonas vaginalis*, Liquid medium, Sensitivity of trichomonas growth

INTRODUCTION

Liquid media are more frequently used for cultivation of *Trichomonas vaginalis* (T.V.) in this country whereas solid media are more common in the European countries and United States, and both the liquid and solid forms present a problem in preservability. Among the widely used in clinical laboratories in this country is Nissui liquid medium with the composition shown in Table 1.

However, this medium can be preserved from a approximately three months and

detectability on it is noticeably diminished with inocula containing only a few trichomonads, and, what is worse, the organism propagates itself on it to a detectable extent only after about ten days of incubation following inoculation. The laboratory examination of a clinical specimen with this medium eventually requires a considerable length of time to yield results for diagnosing the case.

Meanwhile, what is called Asami's liquid medium is most commonly used in many laboratories. Cultures with this liquid medium usually show growth of the organism when the inoculum contains two or more trichomonads, but the medium can be preserved only for a short period and therefore has disadvantage that it should be prepared in the laboratory in order for its application to clinical laboratory diagnosis.

When viewed from a urological standpoint it is of deep significance in laboratory diagnosis to ascertain whether or even a single trichomonad be demonstrated in a given specimen, and even if a single organism is present all in the specimen, it needs to be detected when

Table 1. Commercial medium "Nissui"
(Medium A)

Liver extract (powder)	25.0 g
NaCl	6.5 g
Glucose	5.0 g
Horse serum	80.0 ml
Agar	1.0 g
Cysteine hydrochloridum	0.5 g
Streptomycin	500000 units
Penicillin V	1000000 units
Aq. dil	1000 ml

Divide every 5ml in small glass test tube

cultured. Thus the situation contrasts strongly with that in obstetrics and gynecology where the organisms have diagnostic significance only when present in great numbers.

This report presents the results of laboratory evaluation from the urological viewpoint of a new trial preparation of culture medium for T.V. developed at the Research Laboratory of Nissui Pharmaceutical Co., Ltd., which is expected to have an increased sensitivity of detection with a substantial reduction in duration of incubation.

MATERIALS and METHODS

The experiments were carried out using four strains of T.V. which had been maintained through more than three hundred serial subcultures in Asami's medium after their isolation from male genitourinary specimens. The trichomonads were propagated in a commercial medium (Nissui), hereinafter referred to as medium A, immediately preceding the experiment.

The organisms grown in medium A were collected, washed with physiological saline, resuspended in saline, and counted in a hemocytometer. Appropriate dilutions of the trichomonad suspension were then inoculated onto test media and incubated. The cultures were examined daily for organisms with a microscope. Three types of culture media were tested: the conventional Nissui medium (medium A), a liver extract-free medium with a

new composition (medium B) and Asami's medium. In case of broth containing only 1 or 2 trichomonads, the organisms were collected by means of a glass capillary under the microscope. The cultures were set up with inoculum size ranging widely from 1 to 1×10^4 organisms per tube. Any tube failing to show the organism even in five days of incubation was blind-subcultured serially in Asami's medium up to the second generation for further detection.

RESULTS

The results are summarized in Figure 1, 2, 3.

Inoculated cultures of medium A frequently failed to reveal any appreciable growth of trichomonads; it was particularly frequent in tubes inoculated with ≤ 10 organisms that the organisms were undetectable following incubation, and even those cultures inoculated in the order of 10^2 to 10^4 organisms per tube showed relatively frequent failure in protozoal

Fig. 1. Culture results of medium A

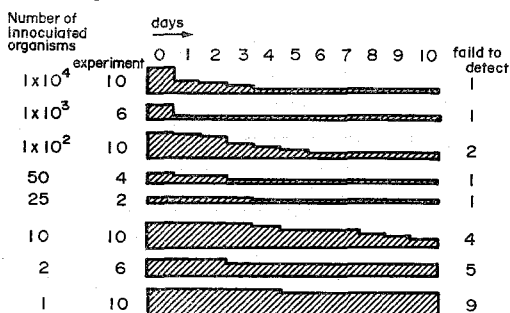


Fig. 2. Culture results of medium B

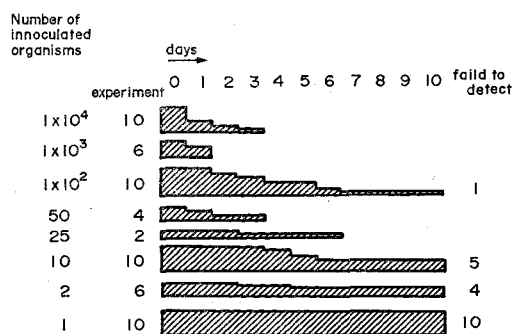
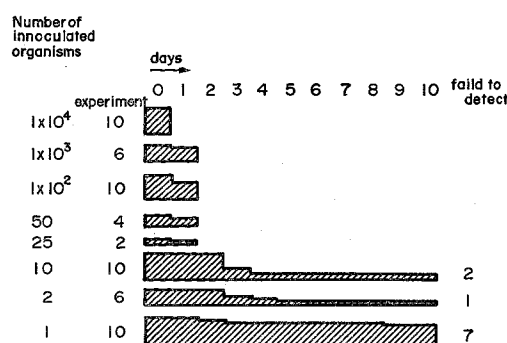


Table 2. New composition medium (Medium B)

Polypeptone	10 g
Yeast extract (powder)	10 g
NaCl	6.5 g
Glucose	5.0 g
Horse serum	80.0 ml
Agar	1.0 g
Cysteine hydrochloridum	0.5 g
Streptomycin	500000 units
Penicillin V	1000000 units
Aq. dil	1000 ml

Divide every 5ml in small glass test tube

Fig. 3. Asamis medium



growth. Even with the large inoculum sizes, the organisms were undemonstrable in the culture within a few days of incubation and became in most instances demonstrable only after the fifth day of incubation.

Table 3. Comparison of sensitivity of diagnostic detection of T.V. among cultures in Medium A and Asami's Medium

Medium		A. (Nissui)	
Asami		pos.	neg.
	pos.	4	13
	neg.	0	—

The data presented in Table 3 show comparison of sensitivity of diagnostic detection of T.V. in clinical specimens among cultures in medium A and Asami's medium. Culture in Asami's medium were always positive for T.V. when inoculated with specimens from patients whose fresh urine deposits revealed the organism, and showed the growth of T.V. even with an inoculum from an occasional case in which the urine deposits were negative for the organism. T.V. was not demonstrable by culture in medium A in some of the cases where urine deposits were positive for this organism; we cannot help assessing medium A as less sensitive in the detection of T.V.

Medium B has not been applied yet in the clinical laboratory, but, experimentally, cultures with this medium frequently failed to demonstrate the growth of tri-

chomonade after inoculation with several tens of organisms per tube and that the length of time of incubation required from demonstrable growth of the organism was only slightly shorter than or virtually comparable to that of culturees with medium A. Although no protozoal enumeration has been made, the cultures with medium B seemed to contain greater numbers of the organisms to facilitate detection more readily. To describe more concretely, three glass slides of fresh preparation were required for accurate demonstration of the organism in the culture of an individual specimen when the examination was carried out with medium A, whereas with medium B, examination of a single slide usually sufficed though three slides were prepared from each culture.

DISCUSSION

The data obtained with Asami's medium shown in Figure 3 eventually are consistent with reports of many investigators. Generally, greater detectability rates were observed with this medium than media A and B; trichomonads were demonstrable, mostly within 4 days of incubation, in 100% of cultures in Asami's medium inoculated with 25 organisms.

It seems reasonable to conclude from the results that:

(1) Medium B has much the same characteristics as medium A, and there is no significant difference in T.V. detectability rate between them.

(2) As compared with media A and B, the use of Asami's medium yields apparently higher rates of T.V. detection.

(3) Medium B may be said to be more advantageous over medium A in that the former has a simplified composition and hence is easier to prepare, with a reduction in cost.

(4) Media A and B cannot be said to meet the requirement to a full extent in the laboratory diagnosis of urological cases.

Kuwahara⁵⁾ and Takemura⁷⁾ appear to have been the first to succeed in cultivation

of T.V. in artificial media. Both investigators cultivated the organism by inoculation of liquid media with vaginal discharges from patients. However, their serial subcultures did not show consistent results, which seemed to indicate some effects of coexisting bacteria.

Most of these methods of cultivation of T.V. before the second world war, were based on the application of Tanabe and Chiba's media for amoebae composed chiefly of serum. Therefore, concurrent bacteria could not be eliminated from the culture and some difficulty was inherent in attempts of serial subculturing of the organism in such media. After the war, the methods of cultivation with addition of small quantities of penicillin and other antibiotics were developed, which have made bacteria-free cultivation practicable. Trussell⁸⁾ and Asami^{1),2)} seem to have been the first to obtain bacteria-free strains of T.V. by such methods.

Bacterial contaminants in the culture of T.V. are rather favorable for the growth of the anaerobic trichomonads and, accordingly, their complete elimination from the culture results in a poor growth of the protozoa. It also seems that the organism not in few instances utilizes bacterial metabolites as a nutrient source. Consequently, the use of anaerobic high-layer fluid media has become increasingly considered and, with the increasing elucidation of the biochemical characteristics of metabolism of the organism, there have been corresponding modifications of culture media which thus have become reliably applicable in the clinical laboratory.

The Asami's medium used in this study was devised in 1954²⁾ and can be applied in the clinical laboratory diagnosis as described above, but it has drawback in respect of preservation. The conventional Nissui medium is a modification of Asami's medium by substitution of horse serum for human serum, elimination of methylene blue, and so forth. The modifications have led to prolongation of the preservation period to three months, and at the same time, to a lowered detective

sensitivity as seen from the tables. Besides, V bouillon³⁾ and glucose-containing VF bouillon⁶⁾ were introduced by Hamada and Magara, respectively, in 1953 for cultivation of T.V.

Almost all of this above-mentioned media are for anaerobic cultivation, while there have been reports of successful propagation of the organism on aerobic media.

Currently, we make it a rule in our laboratory to supplement Asami's medium with streptomycin and kanamycin in addition to penicillin, and in such instance where bacterial contaminants have emerged in the course of serial subculture, to add colimycin and cephalosporins as well to the medium.

Other methods of cultivation, e.g. employing the yolk-sac, have also been introduced although these are not commonly used in the clinical laboratory because of procedural cumbersome^{4,7)}.

It is also common that there have been no laboratory data to demonstrate the lower limit of detective sensitivity of culture on these media, namely, the minimal number of organisms in a given inoculum which unfailingly gives rise to a satisfactory growth in the culture. This might be largely due to the fact that these media were originally designed to facilitate detection of T.V. in vaginal secretions or maintenance of trichomonad survival in vitro in the laboratory and, therefore, such assessment of detective sensitivity has been unnecessary.

The present test, though would seem to be somewhat strict to the media in this view, is considered to be justified when viewed from the urological standpoint.

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和文抄録

新しく作成した液体培地による膣トリコモナス培養結果の評価

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今日、膣トリコモナスの臨床材料からの検出には、市販の培地が使用されているが、その検出感度は必ずしも満足できるものではない。感度のよい培地には保存性がわるいなどの欠点がある。泌尿器科的立場か

らより好ましい培地を追求し、今回新らしく肝エキスを除いた培地について従来の日水培地、浅見培地と比較し、この2種の培地の中間に位置する検出感度であることをたしかめた。